

Supplementary Materials

Table S1. Nucleotide accession numbers of sequences used for *fdhF* primer design (see Fig. 1).

Source	Accession Number ¹
<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> A449	NC_009348.1:1906100-1908244
<i>Aggregatibacter aphrophilus</i> NJ8700	NC_012913.1: c1159571-1157412
<i>Citrobacter koseri</i> ATCC BAA-895 copy 1	NC_009792.1:3531364-3533511
<i>Citrobacter koseri</i> ATCC BAA-895 copy 2	NC_009792.1:1727418-1729565
<i>Citrobacter rodentium</i> ICC168 copy 1	NC_013716.1:c3662542-3660395
<i>Citrobacter rodentium</i> ICC168 copy 2	NC_013716.1:c3568359-3566212
<i>Citrobacter</i> sp. 30_2 copy 1	NZ_GG657366.1:c93031-90884
<i>Citrobacter</i> sp. 30_2 copy 2	NZ_GG657366.1:c1094197-1096347
<i>Citrobacter youngae</i> ATCC 29220 copy 1	NZ_ABWL01000021.1:c93031-90884
<i>Citrobacter youngae</i> ATCC 29220 copy 2	NZ_ABWL01000021.1:c24883-27030
<i>Clostridium bartlettii</i> DSM 16795	NZ_ABEZ02000007.1:c36324-34174
<i>Clostridium beijerinckii</i> NCIMB 8052	NC_009617.1:c4364248-4366389
<i>Clostridium bolteae</i> ATCC BAA-613	NZ_ABCC02000017.1:93731-95716
<i>Clostridium carboxidivorans</i> P7 copy 1	NZ_ACVI01000105.1:231-2378
<i>Clostridium carboxidivorans</i> P7 copy 2	NZ_ACVI01000010.1:36001-38157
<i>Clostridium difficile</i> 630	NC_009089.1:c3884230-3882086
<i>Cronobacter sakazakii</i> ATCC BAA-894	NC_009778.1:c1996280-1998430
<i>Cronobacter turicensis</i> copy 1	NC_013282.1:2002311-2004458
<i>Cronobacter turicensis</i> copy 2	NC_013282.1:1996635-1998845
<i>Dickeya dadantii</i> Ech586	NC_013592.1:2958853-2961003
<i>Dickeya dadantii</i> Ech703	NC_012880.1:c1450903-1453053
<i>Dickeya zeae</i> Ech1591	NC_012912.1:3084906-3087056
<i>Edwardsiella ictaluri</i> 93-146	NC_012779.1:3156478-3158622
<i>Edwardsiella tarda</i> EIB202	NC_013508.1:3053142-3055286
<i>Enterobacter cancerogenus</i> ATCC 35316	NZ_ABWM02000022.1:21042-23189
<i>Enterobacter</i> sp. 638 copy 1	NC_009436.1:c 329787-331934
<i>Enterobacter</i> sp. 638 copy 2	NC_009436.1:c1907448-1909598
<i>Escherichia coli</i> K-12 substr MG1655	NC_000913.2:c4295242-4297389
<i>Escherichia fergusonii</i> ATCC 35469	NC_011740.1:4397249-4399396
<i>Klebsiella pneumoniae</i> NTXH-K2044 copy 1	NC_012731.1:c358869-356722
<i>Klebsiella pneumoniae</i> NTXH-K2044 copy 2	NC_012731.1:3017444-3019594
<i>Pantoea</i> sp. At-9b	NZ_ACYJ01000001:122540-124690
<i>Pectobacterium atrosepticum</i> SCRI1043 copy 1	NC_004547.2:c1752061-1754157
<i>Pectobacterium atrosepticum</i> SCRI1043 copy 2	NC_004547.2:1420602-1422752
<i>Pectobacterium carotovorum</i> sbsp. <i>carotovorum</i> WPP14	NZ_ABVY01000027.1:c9266-11416
<i>Pectobacterium wasabiae</i> WPP163	NC_013421.1:c1930748-1932898
<i>Photobacterium profundum</i> 3TCK	NZ_AAPH01000003.1:97396-99486
<i>Proteus mirabilis</i> HI4320 copy 1	NC_010554.1:3909884-3912028

<i>Proteus mirabilis</i> HI4320 copy 2	NC_010554.1:c3265604-3267772
<i>Providencia alcalifaciens</i> DSM 30120	NZ_ABXW01000042.1:35044-37197
<i>Providencia rustigianii</i> DSM 4541	NZ_ABXV02000023.1:88004-90157
<i>Psychromonas</i> sp. CNPT3	NZ_AAPG01000013.1:c3595-5742
<i>Salmonella enterica</i> sbsp. <i>enterica</i> serovar Typhi CT18	NP_458584; NC_003198.1:4370484-4372631
<i>Salmonella typhimurium</i> LT2	NP_463150; NC_003197.1:c4525350-4527497
<i>Serratia proteamaculans</i> 568	NC_009832.1:c2657681-2659837
<i>Shigella</i> sp. D9	NZ_ACDL01000041.1:c37225-39372
<i>Treponema primitia</i> str. ZAS-2 copy 1 (Sec FDH)	FJ479768:50505-52697
<i>Treponema primitia</i> str. ZAS-2 copy 2 (Cys FDH)	FJ479768:30735-32933
<i>Vibrio angustum</i> S14	NZ_AAOJ01000001.1:c1074316-1076460
<i>Yersinia aldovae</i> ATCC 35236	NZ_ACCB01000002.1:136225-138372
<i>Yersinia bercovieri</i> ATCC 43970	NZ_AALC02000017.1:13658-15805
<i>Yersinia enterocolitica</i> subsp. <i>enterocolitica</i> 8081	NC_008800.1:3050211-3052358
<i>Yersinia frederiksenii</i> ATCC 33641 copy 1	NZ_AALE02000011.1:c133500-135647
<i>Yersinia frederiksenii</i> ATCC 33641 copy 2	NZ_AALE02000004.1:63404-65548
<i>Yersinia mollaretii</i> ATCC 43969 copy 1	NZ_AALD02000005.1:c25400-27571
<i>Yersinia mollaretii</i> ATCC 43969 copy 2	NZ_AALD02000036.1:52-2196
<i>Yersinia rohdei</i> ATCC 43380	NZ_ACCD01000002.1:c116227-118374
<i>Yersinia ruckeri</i> ATCC 29473	NZ_ACCC01000020.1:c42838-44961

¹ A 'c' before genome coordinates indicates complementary sequence

Table S2. PCR primer combinations and concentrations for *fdhF* amplification from pure culture and insect gut templates. Sequenced amplicons were classified as *fdhF*_{Sec} (‘Sec’) or *fdhF*_{Cys} (‘Cys’) versions of *fdhF* based on whether their deduced amino acid translations encode a selenocysteine or cysteine, respectively, at the catalytic active site. All templates are DNA unless noted. Primer set 1: fdhF-F1, fdhF-F2, fdhF-F3, fdhF-R1, fdhF-R2. Primer set 2: universal primers EntfdhFunv- F1, TgfdhFunv-F1, and fdhFunv-R1.

Templates	Primer Combinations (μM final conc.)	Amplicon
<i>Treponema primitia</i> str. ZAS-2	fdhF-F1 (1.0), fdhF-R1 (1.0)	Sec
<i>T. primitia</i> str. ZAS-2	fdhF-F1 (1.0), fdhF-R2 (1.0)	Cys
<i>T. primitia</i> str. ZAS-1	fdhF-F1 (1.0), fdhF-R1 (1.0)	Sec
<i>T. primitia</i> str. ZAS-1	fdhF-F1 (1.0), fdhF-R2 (1.0)	Cys
<i>Buttiauxiella</i> sp. SN-1	fdhF-F3 (1.0), fdhF-R2 (1.0)	Sec
<i>Serratia grimesii</i> str. ZFX-1	fdhF-F2 (1.0), fdhF-R1 (1.0)	Cys
<i>Citrobacter</i> sp. TSA-1	Primer set 2 forward (0.5), reverse (1.0)	Sec
<i>Acetonema longum</i> str. APO-1	Primer set 2 forward (0.5), reverse (1.0)	Sec
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	Primer set 2 forward (0.5), reverse (1.0)	no product
<i>Zootermopsis nevadensis</i> collection ChiA1 gut DNA	Primer set 1 forward (0.3), reverse (0.3)	Sec, Cys
<i>Zootermopsis nevadensis</i> collection ChiA1 gut DNA	Primer set 2 forward (0.5), reverse (1.0)	Sec, Cys
<i>Zootermopsis nevadensis</i> collection ChiB gut cDNA	Primer set 2 forward (0.5), reverse (1.0)	Sec, Cys
<i>Reticulitermes hesperus</i> collection ChiA2 gut DNA	Primer set 2 forward (0.5), reverse (1.0)	Sec, Cys
<i>Incisitermes minor</i> isolate collection Pas1 gut DNA	Primer set 2 forward (0.5), reverse (1.0)	Sec, Cys
<i>Cryptocercus punctulatus</i> gut DNA	Primer set 2 forward (1.0), reverse (1.0)	Sec, Cys

Table S3. FDH phylotype distribution in wood-feeding insect gut inventories. Phylotypes (i.e., operational taxonomic units) were identified using DOTUR at a cutoff of 97% protein similarity level (Jones-Thorton-Taylor corrected). The number of genotypes, inferred based on RFLP sorting, comprising each phylotype are listed. PS1: primers fdhF-F1, fdhF-F2, fdhF-F3, fdhF-R1, fdhF-R2. PS 2: universal primers EntfdhFunv-F1, TgfdhFunv-F1, and fdhFunv-R1.

Clone Library (No. clones, primer set)	Phylotype (No. genotypes)	Abundance
<i>Zootermopsis nevadensis</i> ChiA1 (84, PS 1)	Zn9cys (10)	42.9%
	Zn2cys (1)	22.6%
	Zn70sec (6)	17.9%
	Zn62sec (1)	13.1%
	Zn13cys (1)	1.2%
	Zn51sec (1)	1.2%
	Zn61sec (1)	1.2%
<i>Zootermopsis nevadensis</i> ChiA1 (86, PS 2)	ZnC1cys (2)	45.3%
	ZnD2sec (1)	23.3%
	ZnF7sec (3)	4.7%
	ZnH6cys (2)	3.5%
	ZnB3cys (1)	3.5%
	ZnB5sec (3)	3.5%
	ZnB8sec (1)	2.3%
	ZnC6sec (1)	2.3%
	ZnD3cys (1)	2.3%
	ZnA4cys (2)	2.3%
	ZnC8sec (2)	2.3%
	ZnB9cys (1)	1.2%
	ZnC11cys (1)	1.2%
	ZnE2cys (1)	1.2%
	ZnH8cys (1)	1.2%
<i>Zootermopsis nevadensis</i> ChiB (81, PS 2)	Zn5secRT (2)	29.6%
	Zn16secRT (2)	19.8%
	Zn2cysRT (3)	12.3%
	Zn25secRT (6)	11.1%
	Zn9cysRT (3)	6.2%
	Zn56secRT (2)	3.7%
	Zn55secRT (1)	2.5%
	Zn67cysRT (1)	2.5%
	Zn71cysRT (1)	2.5%
	Zn75cysRT (3)	2.5%
	Zn76secRT (1)	2.5%
	Zn36secRT (1)	1.2%

	Zn51secRT (1)	1.2%
	Zn61secRT (1)	1.2%
	Zn72secRT (1)	1.2%
<i>Reticulitermes hesperus</i> ChiA2 (89, PS 2)		
	Rh36cys (6)	30.3%
	Rh2sec (5)	28.1%
	Rh9sec (1)	10.1%
	Rh15cys (3)	7.9%
	Rh41sec (5)	6.7%
	Rh24sec (2)	5.6%
	Rh35sec (2)	2.2%
	Rh53sec (2)	2.2%
	Rh54cys (2)	2.2%
	Rh47cys (1)	1.1%
	Rh65cys (1)	1.1%
	Rh71sec (1)	1.1%
	Rh93cys (1)	1.1%
<i>Incisitermes minor</i> Pas1 (80, PS 2)		
	Im5cys (5)	18.8%
	Im26sec (3)	17.5%
	Im15sec (5)	16.3%
	Im11cys (7)	13.8%
	Im27sec (2)	10.0%
	Im42cys (2)	6.3%
	Im10sec (2)	5.0%
	Im22sec (1)	5.0%
	Im24cys (2)	2.5%
	Im3sec (1)	2.5%
	Im63sec (2)	2.5%
<i>Cryptocercus puntulatus nymph</i> (136, PS 2)		
	Cp16sec (11)	21.3%
	Cp10sec (12)	17.6%
	Cp3sec (4)	9.6%
	CpF1cys (6)	8.8%
	Cp14sec (4)	8.1%
	Cp72cys (4)	4.4%
	CpH1cys (2)	3.7%
	CpD8sec (1)	2.9%
	CpB3sec (3)	2.9%
	Cp9cys (2)	2.9%
	CpB10sec (1)	2.9%
	CpC3sec (2)	2.9%
	CpC1cys (1)	1.5%
	CpD1cys (1)	1.5%
	CpB2sec (1)	1.5%
	CpE8cys (1)	1.5%
	CpF9cys (1)	1.5%
	CpF8cys (1)	1.5%
	Cp28sec (1)	1.5%
	Cp24sec (1)	1.5%
	Cp34sec (1)	1.5%

Cp78sec (1)	1.5%
Cp82sec (1)	1.5%
Cp94sec (1)	1.5%

Table S4. Summary of *fdhF* inventories generated from wood-feeding insect guts.

Clone library templates	Sample Type	No. Clones Analyzed	No. of OTU ¹	Mean Chao1 (SD) ²	95% LCI, HCI ³	No. Sec, Cys OTU ⁴
<i>Zootermopsis nevadensis</i> collection ChiA1	DNA	84	7	7.53 (1.88)	6.61, 17.23	4, 3
<i>Zootermopsis nevadensis</i> collection ChiA1	DNA	86	15	14.96 (2.78)	13.11, 27.81	6, 9
<i>Zootermopsis nevadensis</i> collection ChiB	cDNA	81	15	14.78 (2.52)	13.20, 26.79	10, 5
<i>Reticulitermes hesperus</i> collection ChiA2	DNA	89	13	13.66 (3.86)	11.49, 33.15	7, 6
<i>Incisitermes minor</i> isolate collection Pas1	DNA	80	11	10.92 (0.62)	10.69, 13.6	7, 4
<i>Cryptocercus punctulatus</i> nymph ⁵	DNA	136	24	21.52 (2.97)	21.52, 37.03	15, 9

¹ Number of operational taxonomic units (OTU) determined using DOTUR (Schloss and Handelsman, 2005) based on > 3% amino acid distance between different phylotypes.

² Bias-corrected Chao1 diversity estimator calculated using EstimateS (Colwell, 2009) based on 100 randomizations, sampling without replacement; SD = standard deviation.

³ Lower (LCI) and higher (HCI) 95% confidence interval limits for mean Chao1 as calculated by EstimateS.

⁴ Number of unique Sec and Cys FDH_H phylotypes.

⁵ Sequences derived from PCR at different annealing temperatures (51°C and 57 °C) were combined for analyses.

Table S5. Amino acid alignment in the area of a characteristic amino acid indel (bold) found only in Gut spirochete group FDH_H sequences. The alignment corresponds to amino acids 394-420 in the selenocysteine encoding FDH_H of *T. primitia* str. ZAS-2. When compared to the protein crystal structure of *E. coli* FDH_H (protein databank identifier 1aa6), the indel was predicted to be located next to an α -helix and hydrogen-bonded turn on the exterior of FDH_H. Sequences are listed in phylogenetic order (see Figure 6).

Sequence	Amino Acid Alignment
Zn16secRT	LSDQPGITLTLVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
ZnB8sec	LSDQPGITLTLVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Zn72secRT	LSDQPGITLTLVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Zn51sec	LSDQPGITLTLVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Zn5secRT	LSDQPGITLTLVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Zn56secRT	LSDKPGITLTLVPHHVL HEKD PTKQIHAYYIMGEDPGQSDPD
ZnB5sec	LSDKPGITLTLVPHHVL HEKD PTKQIHAYYIMGEDPGQSDPD
Zn25secRT	LSDKPGITLTAVPHQVL HEKD PAKQIHAYYIMGEDPGQSDPD
ZnF7sec	LSDKPGITLTAVPHQVL HEKD PAKQIHAYYIMGEDPGQSDPD
ZnC6sec	LSDKAGITLTLVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Cp94sec	LSDQLGITLTTVPHHVL HEKD PKKRIHAYYIMGEGPGQSDPD
Cp14sec	LSDQPGITLTVVPHQVL HEKD PAKQIHAYYIMGEDPGQSDPD
CpB3sec	LSDQPGITLTVVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Cp34sec	LSDQPGITLTVVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Cp82sec	LSDQPGITLTVVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Cp10sec	LSDQPGITLTVVPHHVL HEKD PAKQIHAYYIMGEDPGQSDPD
Rh71sec	LSPDVGITLTTVPHQVL HETD PKKKIHAYYIMGEDPAQSDPD
Rh9sec	LSPDVGITLTTVPHQVL HETD PKKQIHAYYIMGEDPAQSDPD
Rh24sec	LSADIGITLTTVPHQVL HEKD PKKQIHAYYIMGEDPGQSDPD
Zn36secRT	LSDQPGITLTTVPHQVL HETD PRKQIHAYYIMGEDPGQSDPD
Zn51secRT	LSPDLGITLTTVPHQVL HEKD PKKQIHAYYIMGEDPGQSDPD
Zn61sec	LSPDLGITLTTVPHQVL HEKD PKKQIHAYYIMGEDPGQSDSD
Cp16sec	LSDKLGITLTTVPHQVL HETD PTKQIHAYYIMGEDPGQSDPD
Cp3sec	LSDKAGITLTMVPHQVL AEKD PAKKIHAYYIMGEDPGQSDPD
CpC3sec	LSDKAGTTLTMVPHQVL AE TDPAKKIHAYYIMGEDPGQSDPD
Cp24sec	LSDKPGITLTMVPHQVL AE TDPAKKIHAYYIMGEDPGQSDPD
Cp78sec	LSDKPGITLTMVPHQVL AE TDPAKKIHAYYIMGEDPGQSDPD
<i>Treponema primitia</i> str. ZAS-2 fdhFsec	LSDKPGITLTVVPHHVL HEKD PTKQIHAYYIMGEDPVQSDPD
ZnC8sec	LSDKPGITLTVVPHHVL HEKD PTKQIHAYYIMGEDPVQSDPD
Zn61secRT	LSDKPGITLTVVPHHVL HEKD PTKQIHAYYIMGEDPVQSDPD

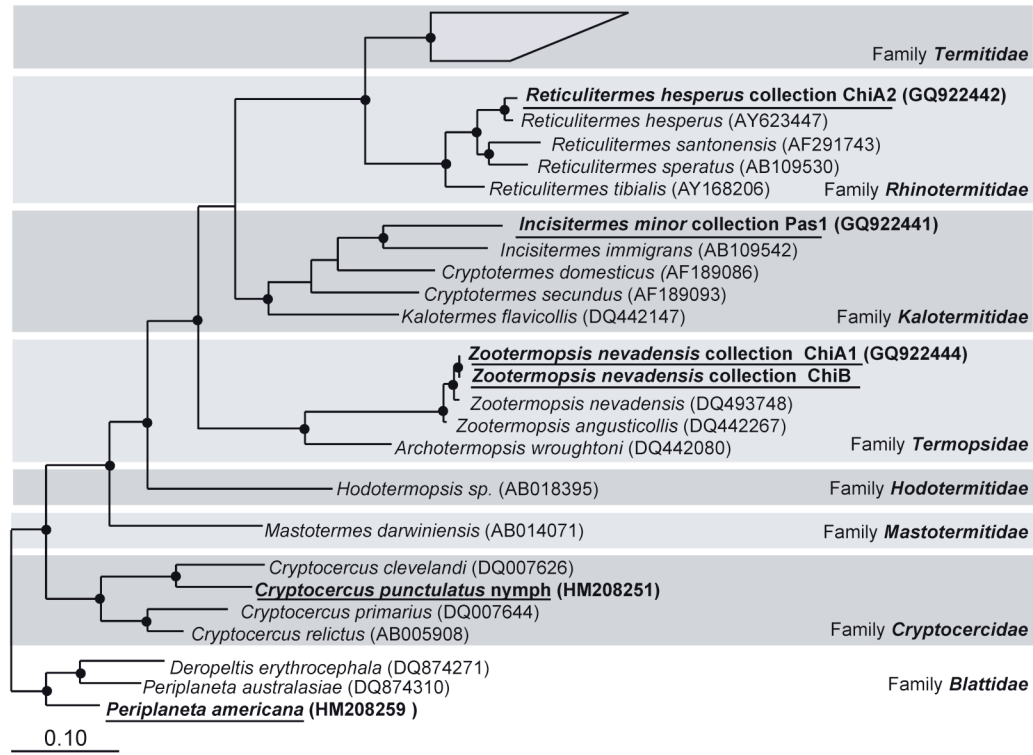
Zn70sec	LSDKPGITLTVVPHHVL HEKD PTKQIHAYYIMGEDPVQSDPD
<i>Treponema primitia</i> str. ZAS-1 fdhFsec	LSDKPGITLTVVPHHVL HETD PAKQIHAYYIMGEDPVQSDPD
CpD8sec	LSDQAGITLTVVPHHVL HEKD PAKQIHAYYIMGEDPVQSDPD
Zn55secRT	LSDKAGITLTVVPHQVL HEKD PKKQIHAYYIMGEDPVQSDPD
Rh41sec	LSDQPGITLTVVPHHVL HETD PAKQIHAYYIMGEDPAQSDPD
Im22sec	LSGEPGITLTVVPHHVL HEKD PAKHIRAYYVMGEDPAQSDPD
Im26sec	LSDQPGITLTMVPHQVL HEKD PAKKIRGYIMGEDPAQSDPD
Im27sec	LSDQPGITLTMVPHHVL HEKD PAKQIHAYYVLGEDPAQSDPD
Im63sec	LSDQPGITLTMVPHHVL HEKD PAKKIRAYYIMGEDPAQSDPD
Im10sec	LSDQAGITLTGVPHQVL HETD PAKKIRAYYIMGEDPAQSDPD
Rh2sec	LPDQNGITLTVVPHQVL HEKD PTKQIHGYIMGEDPVQSDPD
Rh35sec	LPDQNGITLTVVPHQVL HETD PAKKIHGYIMGEDPVQSDPD
Rh53sec	LPAENGITLTVVPGRVL HEKD PSKQIHAYYIMGEDPVQSDPD
Zn62sec	LSNKIGIPLTQVPHYVL HETE -EKKIRAYYIFGEDPAQSDPD
ZnD2sec	LSNKIGIPLTQVPHYVL HETE -EKKIRAYYIFGEDPAQSDPD
Zn76secRT	LSNKIGIPITQVPRYVL HEPE -EKKIRAYYIFGEDPAQSDPD
Im3sec	LPEKVGIPLTQVPHYVL HEPE -ERKIRAYYVFGEDPAQSDPD
CpB2sec	LSDKVGITLTKVPHHVL HEKG -AKKIHAYYIMGEDPAQSDPD
Im15sec	LSDKVGCPIPTHVPHRVL HEKD PAKRIHAYYIFGEDPAQSDPD
ZnC1cys	LDNKVGIQLTRIPFV HEKN PANRIHAYYITGEDPAQSDPD
ZnHcys	LDNKVGIQLTRIPFV HEKN PANRIHAYYITGEDPAQSDPD
CpE8cys	LDNKVGIQLTRIPFV HEQD PAKRIHAYYITGEDPAQSDPD
Rh15cys	LDNKVGIQLTRIAEFT HQKD PAKRIHAYYITGEDPAQSDPD
Rh54cys	LDNKVGIQLTRIAEFT HQAD PAKRIHAYYITGEDPAQSDPD
Zn13cys	LDNKVGVQLTRIPELVL HEKD PAKRIHAYYITGEDPAQSDPD
Rh36cys	LDNKVGIQLTRIPELV HEKD PAKRIHAYYITGEDPAQSDPD
Rh47cys	LDNKVGIQLTRIPFVL HEKD PAKRIHAYYITGEDPAQSDPD
Rh65cys	LDNKVGIQLTRIPFV HEKD PAKRIHAYYITGEDPAQSDPD
Zn71cysRT	LDNKVGLQLTRVPEFVL QEKD PAKQIHAYYITGEDPAQSDPD
ZnD3cys	LDNKVGLQLTRVPEFVL HEKD PAKCIHAYYITGEDPAQSDPD
Zn75cysRT	LDNKVGIQLTRVPEFVL HEKD PKKQLHAYYITGEDPAQSDPD
ZnA4cys	LDNKVGIQLTRVPEFVL HEKD PKKQLHAYYITGEDPAQSDPD
Cp72cys	LDNKVGIQLTRVPEFV HEKD PAKRIHAYYITGEDPAQSDPD
CpF8cys	LDNKVGIQLTRVPEFV HEKD PAKRIHAYYITGEDPAQSDPD
CpF9cys	LDNKVGIQLTRVPEFV HDKD PAKRIHAYYITGEDPAQSDPD
CpC1cys	LDNKVGIQLTRVPEFV HEKD PAKRIHAYYITGEDPAQSDPD
CpH1cys	LDNKVGIQLTRVPEFV HEKD PAKRIHAYYITGEDPAQSDPD
CpD1cys	LDNQVGLQLTRVPEFVL HEKD PKKRIHAYYITGEDPAQSDPD
CpF1cys	LDNKVGIQLTRVPEFV HEKD PAKRIHAYYITGEDPAQSDPD
Im11cys	LDDKVGILQLTRVPEFV QHMSD PAKRLHAYYITGEDPCQSDPD
Im42cys	LDDKAGIQLTRVPEFV QHESD PAKRIHAYYITGEDPCQSDPD
<i>Treponema primitia</i> str. ZAS-2 fdhFcys	LSNKAGIQLTRVPEFV HEKD PAKRIHAYYITGEDPAQSDPD
ZnH6cys	LSNKVGLQLTRVPEFV HEKD PAKRIHAYYITGEDPAQSDPD

Zn9cys	LSNKAGIQLTRVPEFVI HEKDP AKRIHAYYITGEDPAQSDPD
Zn9cysRT	LSNKAGIQLTRVPEFVI HEKDP AKRIHAYYITGEDPAQSDPD
Zn67cysRT	LSNKAGIQLTRVPEFVI HEKDP AKRIHAYYITGEDPAQSDPD
ZnB9cys	LSNKAGIQLTRVPEFVI HEKDP AKRIHAYYITGEDPAQSDPD
<i>Treponema primitia</i> str. ZAS-1 fdhFcys	LSNKAGIQLTRVPEFVI HEKDP AKRIHAYYITGEDPAQSDPD
Zn2cys	LSNKVGIQLTRVPEFVI HEKDP AKRIHAYYITGEDPAQSDPD
Zn2cysRT	LSNKVGIQLTRVPEFVI HEKDP AKRIHAYYITGEDPAQSDPD
ZnB3cys	LSNKVGIQLTRVPEFVI HEKDP AKRIHAYYITGEDPAQSDPD
Im24cys	LSDKVGLALTRVPERVL HEEDP AKRIHAYYIFGEDPGQSDPD
Im5cys	LSDKVGLALTRVPERVL HEEDP AKRIHAYYIFGEDPGQSDPD
Cp9cys	LPPEVGLQLTRVPEKVI HEKDP AKRIHAYYIFGEDPAQSDPD
Rh93cys	LPDQPGQLTRVPERVV HEKDP AKQIHAYYIFGEDPAQSDPD
ZnC11cys	LSPAVGLHVTRVPEFVL DPPEE AKRIHAYYVYGEDLAHSDPN
ZnH8cys	LSPTVGLHVTRVPEFVL KEPDP AKQIHAYYVYGEDPAHSDPN
CpB10sec	LSPNAGLHVTRVPEHVL EPSP EKAIGHYYVYGEDPAHSDPN
<i>Clostridium bartlettii</i> DSM 16795	LPSKVGLKLTevPHAVL E ----EHKIKAYYIFGEDPVQSDPD
<i>Clostridium difficile</i> 630	LSPNNGYSLTQVPNLVL K ----EKKLKAYYIFGEDPVQSDPD
<i>Clostridium beijerinckii</i> NCIMB 8052	LSDKNGYFLTQVPELVL K ----EDKIKAYYIFGEDPVQSDPN
<i>Clostridium carboxidivorans</i> P7 copy 2	LSDKVGYHLTEVPKLV LK ----ENKLKAYYIMGEDTVQSDPN
<i>Clostridium carboxidivorans</i> P7 copy 1	LPNKVGYHLTEVPHLV LK ----EDKIKAYYIMGEDPVQSDPD
<i>Acetonebma longum</i> APO-1	LPAKPGYHLTEVPHLAR-----EGKIKAYYIFGEDPVQSDPD
<i>Citrobacter koseri</i> ATCC BAA-895 copy 1	LPAHTGYRISELPHRAA-----HGEVRAAYIMGEDPLQTDAE
<i>Escherichia coli</i> K-12	LPAHTGYRISELPHRAA-----HGEVRAAYIMGEDPLQTDAE
<i>Buttiauxiella</i> SN1	LPAHTGYRISELPHRVA-----HGEVYAAAYIMGEDPLQTDAE
<i>Proteus mirabilis</i> HI4320 copy 1	MPPEVGYALSEVPHNID-----HGLIKAHYVMGEDPLQTEPD
Cp28sec	NSREKGYPLSELPHNAI-----SGKVKAFYVMGEDPMTQTEPD
<i>Yersinia frederiksenii</i> ATCC 33641 copy 2	LPAHVGYSITDVPHKVA-----EGKLKAYYVFGEDPIQTEPD
ZnE2cys	FPEKVGLHLTEVPHAVH-----EGKLKAFYIMGEDPLQTEPD
<i>Yersinia frederiksenii</i> ATCC 33641 copy 1	LSGKIGYSLTDVPHKVK-----EGKIKANYVMGEDPLQTEPD
<i>Citrobacter koseri</i> ATCC BAA-895 copy 2	MDDKVGTRITEVPHLAM-----EGKIKAYYIMGEDPLQTEAD
<i>Proteus mirabilis</i> HI4320 copy 2	LDPQVGYRITEVPHLAI-----EGKVKAYYIMGEDPLQTEAD

Figure S1. Mitochondrial cytochrome oxidase II phylogeny of insects representing major termite (*Mastotermitidae*, *Hodotermitidae*, *Termopsidae*, *Kalotermitidae*, *Rhinotermitidae*, *Termitidae*), wood-feeding roach (*Cryptocercidae*), and omnivorous roach (*Blattidae*) families. *Serritermitidae*, a rare group of lower termites from Brazil, is usually classified as a seventh termite family (Krishna, 1970; Grimaldi and Engel, 2005). Insects examined in this study are underlined. Families in which the wood-feeding ability has been well-established are highlighted by shaded boxes. Members of the first 5 termite families are classified as “lower” termites; those within the *Termitidae* are “higher” termites. 11 cytochrome oxidase sequences (*Amitermes dentatus* acc. no. DQ442065, *Amitermes evuncifer* DQ442066, *Cornitermes pugnax* DQ442106, *Cornitermes walkeri* AB005577, *Labiatermes labralis* DQ442149, *Microcerotermes newmani* DQ442166, *Microcerotermes parvus* DQ442167, *Nasutitermes corniger* AB037327, *Nasutitermes ephratae* AB037328, *Nasutitermes* sp. Warnecke-2007 EU236539, *Nasutitermes nigriceps* AB037329) comprise the grouped clade *Termitidae*. The tree was calculated based on 393 aligned nucleotides using the maximum likelihood algorithm AxML. Filled circles indicate nodes supported by three different tree construction methods (Fitch distance, Phylip DNA parsimony, and AxML). The scale bar represents 0.1 nucleotide changes per alignment position.

Figure S2. Rarefaction curves calculated using EstimateS for each insect gut *fdhF* DNA or cDNA inventory. Sequences were first binned into operational taxonomic units at a cutoff of 97% amino acid similarity (Jones-Thorton-Taylor corrected 3% amino acid difference) using DOTUR. Inventory templates (Zn, *Zootermopsis nevadensis*; Rh, *Reticulitermes hesperus*; Im, *Incisitermes minor*; Cp, *Cryptocercus punctulatus*) and primer sets (PS1, primer set 1; PS2, primer set 2) are designated in the upper left corner. Primer set definitions are listed in Table S2.

Sup. Fig. 1:



Sup. Fig. 2:

